CYTOLOGICAL STUDIES ON POLYPLOIDS DERIVED FROM TETRAPLOID *FRAGARIA VESCA* L. AND CULTIVATED STRAWBERRIES

By

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INTRODUCTION

By using a colchicine induced (4) autotetraploid Fragaria vesca L. in a series of crosses with the octoploid cultivated strawberry (X F. ananassa Duch.), hybrids have been obtained varying in fertility from completely sterile types to fully fertile ones. Some of the fertile seedlings have a pleasing, high aroma similar to that of F. vesca L. Since aroma is a desirable character to incorporate into cultivated strawberries, the fertile new types may be of economic importance.

Previous workers have obtained hybrid seedlings of diploid F. vesca x cultivated strawberry, but they were always sterile so that new types never became established. Since the hybrids of tetraploid F. vesca x cultivated strawberry when backcrossed to the cultivated strawberry, gave progenies with both fertile and infertile forms, questions arose regarding the chromosome numbers of these plants, their meiotic behavior, their pollen production, and the procedure to use in a practical breeding program. Consequently, a cytological study dealing with this material seemed necessary to answer the above questions and to serve as a basis for further hybridization work. This is a report of such a study.

LITERATURE REVIEW

A comprehensive review of literature on the strawberry breeding work done up to 1937 was given by Darrow (3). Hence, only those papers that are pertinent to the present cytological report will be considered here. Duchesne (6) in 1766 accurately described many different species of strawberries. The chromosome numbers of the different species were

unknown until Ichijima (11), Kihara (13), and Longley (17) reported on them in 1926. Longley (17) pointed out that there were three naturally occurring groups: Diploid, hexaploid, and octoploid with a base number of X=7. He considered F. vesca one of the most primitive species. The diploid species with 14 somatic chromosomes are represented today by F. vesca L., F. nilgerrensis Schlecht., F. daltoniana Gay, and F. viridis Duch.; there is only one hexaploid: F. moschata Duch., but there are three octoploid species: F. virginiana Duch., F. chiloensis (L.) Duch., and F. ovalis (Lehm.) Rydb. The cultivated strawberry (X F. ananassa Duch.) is believed to have resulted from hybridization of F. virginiana and F. chiloensis as first noted by Duchesne (6).

Previous reports have shown that crosses between Fragaria species of different chromosome numbers have given sterile or only slightly fertile hybrids, but interspecies crosses made within any one chromosome group usually have given fertile plants. Under natural conditions apparently the same situation prevails. Bunyard (2) in a detailed history of the strawberry dating from about the 13th century pointed out that the European (F. moschata) and North American (F. virginiana) species remained unchanged when grown in the same vicinity.

Mangelsdorf and East (18), Yarnell (25), Powers (20), and others have shown that species with the same chromosome numbers cross readily and that irregularities in meiosis are infrequent and of a minor nature. Ichijima (11) concluded from his studies that meiosis within the different species was very regular. Similarly, the meiotic behavior of hybrids derived from crosses between species within a chromosome group was regular,

with the exception of a cross between two diploid species, F. bracteata Heller x F. Helleri Holz. (allied to F. vesca) which gave a single tetraploid plant. In a later study Ichijima (12) reported many meiotic irregularities in pentaploids derived from crosses of diploid x octoploid species. All of his attempts to cross diploid x hexaploid or hexaploid x diploid yielded only seedlings with the maternal chromosome number, all apparently coming from self pollinations. Crosses of hexaploid x octoploid species failed to set any fruit. Yarnell (26, 27) conducted extensive cytological studies of strawberry crosses. He obtained pentaploids (2n=35) from the cross of F. vesca x F. chiloensis. One of the pentaploids set open pollinated seeds which produced two plants, one with 70 chromosomes and one with 90. He used tetraploids obtained from the plant described by Ichijima in crosses with other species. Hexaploid x tetraploid crosses and the reciprocal gave 16 plants, all of which were entirely sterile. Seedlings from octoploid x tetraploid crosses were completely sterile except for one plant that set some seed. Aneuploid seedlings were obtained in some cases. His meiotic studies (25) of a triploid showed that quadrivalents, trivalents, and bivalents occurred and yet the plants were slightly fertile.

Lilienfeld (15) reported the origin of a fertile tetraploid (2n=28) obtained from F. nipponica Makino (2n=14) x F. elatior [F. moschata] (2n=42). The reciprocal cross was difficult to make and gave pentaploids (2n=35) instead of the expected tetraploids -- presumably from unreduced F. nipponica pollen fertilizing reduced F. elatior ovules. In a selfed population of 319 plants of the tetraploid, 1 plant was larger, more fertile than the others, and had 42 chromosomes -- arising presumably

from an unreduced gamete with 28 and a reduced gamete with 14 chromosomes. Later Lilienfeld (16) reported that this plant with 42 chromosomes when crossed with F. elatior (2n=42) produced fertile seedlings all with 42 chromosomes. She postulates, therefore, that F. elatior is an autohexaploid derived from F. nipponica or closely related diploid species.

Naturally occurring tetraploid species were unknown until 1934 when Fedorova (7) reported that Petrov found a form with 28 chromosomes he referred to as F. orientalis Los., an Asiatic species previously considered a botanical variety of F. vesca. Fedorova (7) obtained 15 seedlings from 5,430 seed of the cross F. vesca x F. elatior [Moschata], 11 of which were maternal diploids, 2 tetraploids and 2 pentaploids. The tetraploids resembled F. vesca more than F. elatior in appearance and were completely sterile. One pentaploid was partially fertile, but the other was sterile. A cross of cultivated strawberry x F. elatior yielded 45 seedlings of which 5 were partially fertile. One seedling with 49 chromosomes was selfed and in the progeny, 14 plants were obtained that had 42, 56, 63, 77, 84, and 98 chromosomes, indicating that unreduced gametes frequently occurred. In a cross of F. vesca x F. moschata, Schiemann (21) obtained maternal types and 6 true hybrids of which 1 was tetraploid and 5 were pentaploid. The pentaploids apparently resulted from 2 genomes of F. vesca (unreduced F. vesca) and 3 genomes of F. moschata. Nilsson and Johansson (19) used a colchicine induced tetraploid F. vesca in crosses with an octoploid and obtained hexaploid plants which were sterile. Attempts to double the pentaploid obtained from diploid F. vesca x cultivated octoploid were unsuccessful.

Extensive interspecies hybridization has been conducted in Russia (5, 7) and recent reports by Fedorova (8, 9) and Smolyaninova (22) have been made since the present study was initiated. Although their polyploid material was derived largely from the original cross of cultivated strawberries x F. moschata, their results closely parallel certain phases to be reported here. Smolyaninova (22) reported that most of the seedlings from a cross of F. moschata x cultivated strawberry were sterile and vigorous, but a few were fairly fertile with good aroma. Their appearance was similar to the cultivated strawberry, although the plants examined cytologically had the expected number of 49 chromosomes. Fedorova, in a later publication (9) reported on a partially fertile plant from the cross, cultivated strawberry x F. moschata. When this plant was selfed, the progeny had seedlings with chromosome numbers of 2n=42, 56, 63, 70, 77, 84, and 98. The 63 and 70 chromosome plants were most vigorous whereas those with 98 were dwarfs. The fertility of the hybrids varied from completely sterile to fully fertile plants within each chromosome group of 42, 56, 63, 70, and 77. Meiotic studies indicated that reduced fertility was associated with the presence of univalent and multivalent configurations. Multivalent association seemed to cause greater reduction in fertility than did univalents so that in the 63 chromosome group, the fertile plants had no multivalents but had a preponderance of bivalents with a few univalents. Waldo and Darrow (23) grew seedlings of F. virginiana x F. moschata and although they were vigorous, only 7 out of a large population were partially fertile. All of the seedlings obtained by Kihara (14) from a similar cross were sterile.

The chromosomes in strawberry are very small in both somatic and meiotic cells as pointed out by previous investigators. Yarnell (24) measured the chromosomes in root tip cells in diploid species and found them to have the following lengths in microns: 0.9, 1.0, 1.2, 1.3, 1.4, 1.5, and 1.7

It is apparent from the results obtained by these previous workers that hybrids from crosses between species of different chromosome numbers have been sterile or only slightly fertile. Thus none of the characters from F. vesca or F. moschata have ever been incorporated by controlled hybridization into F. virginiana, F. chiloensis or their cultivated counterpart, X F. ananassa, with the possible exception of Fedorova's recent hybrid forms.

MATERIALS AND METHODS

Materials

The tetraploid F. vesca obtained by Dermen and Darrow (4) was used as a source of F. vesca characters. From a cross made in 1940, using a pistillate cultivated strawberry (US 1798) as the seed parent and the tetraploid F. vesca as the pollen parent, hybrids were obtained, most of which were sterile or only slightly fertile. In 1943 four of these slightly fertile F_1 plants were planted in a field with a large collection of cultivated varieties. Open pollinated seeds were collected from the hybrid selections and used to produce progenies from which were obtained in 1946 both fertile and infertile plants. Some of the above-mentioned selections were used as parents in crosses in 1947 and some of the earlier crosses were repeated so that the chromosome numbers of

the plants in the various progenies could be determined. The crosses made and progenies examined cytologically are listed in Table 2.

Methods

Crosses were made in the greenhouse in early spring, the seeds harvested from fully ripened fruits, and kept dry in the laboratory until they were planted on shredded sphagnum about July 1. The seedlings were spotted off into 4" pots about September 1 and most of the seedlings grew vigorously so that it was relatively easy to get good root tips for chromosome counts. After root tip samples were obtained, the plants were placed in a cool greenhouse so that flower bud initiation could take place. They were left in the greenhouse the next spring and flowering and fruiting records taken.

The plants were classified for fertility by placing them in one of the following 5 groups: (1) plants with no flowers, (2) plants sterile, producing flowers but not setting seeds, (3) plants slightly fertile with "nubbin" fruits having only a few seeds, (4) partial fertility in which the berry filled out and had a number of seeds, and (5) complete fertility in which the berries were well developed and had a relatively full complement of seeds.

Pollen grains were stained with aceto-carmine to determine the aborted pollen.

The paraffin method was used for the preparation of nearly all the cytological material. The material was fixed in Navashin's or Graf's fluid, embedded, sectioned at 10 to 12 microns, and stained in crystal violet, using a modified technique in which the material was kept in the stain for 18 to 24 hours and then destained in 95% alcohol.

Bamford (1) had found this a desirable method to use with gladiolus root tips. The slides were cleared in xylol, mounted in clarite and examined with an oil immersion 90x objective.

Photomicrographs were made with a Bausch and Lomb type K 3 1/4 x 4 1/4 camera, using contrast process panchromatic cut film.

RESULTS

Chromosome numbers of first polyploid selections

Since the chromosome numbers of the selections made in 1943 and 1946 were unknown when the present studies were initiated in 1947, they were determined as a first step in the work and they are given in Table 1 along with the chromosome numbers of the parents.

TABLE 1. Chromosome numbers of the first polyploid strawberry selections and their parents

Selection	Parentage of selection	Chromosome number of parents	Chromosome number of selection
F. vesca 4x	*F. vesca* 2x	14 + 14	28
3502	US 1798 X *F. vesca* 4x	56 X 28	42
3504	US 1798 X *F. vesca* 4x	56 X 28	42
3504-2-1	3504 X 8x A.V.[1/]	42 X 56	49
3266-1-1	3266 X 8x A.V.	42 X 56	70
3266-1-2	3266 X 8x A.V.	42 X 56	70
3267-1-1	3267 X 8x A.V.	42 X 56	70
3502-1-1	3502 X 8x A.V.	42 X 56	70
3504-2-2	3504 X 8x A.V.	42 X 56	70
3504-2-4	3504 X 8x A.V.	42 X 56	70

1/ Assorted varieties. See page 6 of text for explanation.

The tetraploid F. vesca is one of the parents and a cultivated variety the other in each of the hybrid hexaploids: 3502, 3504, 3266, and 3267. Only the 3502 and 3504 of these 4 selections were still in existence in 1947 for cytological examination but both had 42 chromosomes which is the expected intermediate number of the parents and, from the breeding behavior of the other two, it would appear that they also had 42 chromosomes. The occurrence of plants with 70 chromosomes (decaploids) obtained by natural pollination of the hexaploids (2n=42) by the cultivated strawberry (2n=56) is significant (Table 1). The fact that these types are fertile makes them of particular interest, especially since the one heptaploid selection (3504-2-1) from the same cross is only partially fertile. The 1 selection with 49 chromosomes and 6 with 70 chromosomes were the only seedlings saved out of a population of about 300 mature plants obtained from seeds of hexaploids x octoploid varieties. Chromosome numbers were not determined for any of the plants discarded from the population, unfortunately, as it was assumed that all the plants had 49 chromosomes.

Crosses made in 1947

The controlled crosses made and results obtained from the pollinations in 1947 are given in Table 2. Relatively few flowers were used in each cross as the primary interest was to obtain seedlings for progeny studies rather than a study of cross pollination relationships. Despite the limited nature of the data a few interesting relationships are evident. One is that the hexaploid 3504 produced very few seeds per berry when cross pollinated and 3502 practically none when selfed although its pollen was

TABLE 2. Strawberry crosses in 1947 involving polyploid selections

Cross	Chromosome number of parents	Flowers pollinated: No.	Flowers set: No.	Seeds obtained: No.	Seedlings obtained: No.	%
Midland X *F. vesca* 4x	56 X 28	15	12	160	56	35
Md. 683 X 3502	56 X 42	17	10	106	42	40
3504 X Midland	42 X 56	47	36	100	24	24
3502 X self	42 X 42	50	1	2	0	0
Midland X Fairpeake	56 X 56	20	20	1800	--	--
3266-1-1 X 3502-1-1	70 X 70	5	3	107	14	13
3266-1-1 X Midland	70 X 56	10	10	161	19	12
3266-1-2 X 3502-1-1	70 X 70	3	3	55	--	--
3266-1-2 X Midland	70 X 56	6	6	411	--	--
3504-2-2 X 3502-1-1	70 X 70	6	6	404	89	22
3504-2-2 X Midland	70 X 56	4	4	497	208	42
F. vesca 2x X Midland	14 X 56	3	3	80	2	2.5

functional when used on an octoploid. When the decaploids (2n=70) were pollinated by either another decaploid or an octoploid, the number of seeds obtained was about the same. Seed germination was low for most lots and, consequently, the progenies for later studies were not so large as would be desirable. However, it should be noted that successful crosses were made readily between different chromosome number plants and seedlings were produced from all crosses attempted except the 3502 x self, as indicated by the data in Table 2.

Chromosome numbers of seedlings from different crosses and their fertility

The chromosome numbers of the seedlings in different crosses are given in Table 3. Strawberries are difficult to study cytologically as the chromosomes are very small, numerous and tend to overlap.

TABLE 3. Chromosome numbers of strawberry seedlings from different crosses as determined by somatic counts

Crosses	Chromosome numbers of parents	Chromosome numbers of seedlings in the progenies												
		40-42	42	42-47	47-49	49	49-56	56	56-61	61-63	63	63-67	67-70	70-77	Total
Midland X *F. vesca* 4x	56 X 28	18		8			8								34
Md. 683 X 3502	56 X 42				16		7	8					3	1	35
3504 X Midland	42 X 56				6		14						2		22
3504 X 8x A.V.	42 X 56				8		2						1		11
3504-2-2 X Midland	70 X 56								26	9					35
3504-2-2 X 3502-1-1	70 X 70										15		4		19
3266-1-1 X 3502-1-1	70 X 70										1		11		12
3267-1-1 X 3502-1-1	70 X 70										25		5		30

In somatic tissue one rarely finds figures in which all the chromosomes are distinctly separated and consequently exact counts could not be obtained for all plants. Where excellent figures were found for counting, the numbers were always multiples of the basic number X=7. It may be reasonably assumed, therefore, that of the 18 seedlings listed in Table 3 as having 40 - 42 chromosomes, all had 42, the intermediate number of the parents. Figures 1 to 9 are photomicrographs of the different chromosome numbers in somatic cells.

The fertility was recorded of the seedlings whose chromosome numbers had been determined in the different crosses, and the data is summarized in Table 4. A few seedlings were too weak to live over winter so that there are fewer seedlings listed for some crosses in Table 4 than in Table 3.

TABLE 4. Fertility ratings of strawberry seedlings of different chromosome numbers derived from various crosses

Crosses	Chromosome number of plants	Number of plants in the classes					
		No flowers	Sterile flowers	Slightly fertile	Partially fertile	Fertile	Total
Midland X *F. vesca* 4x	42	13	12	1			26
Md. 683 X 3502	49	3	7	13			23
Md. 683 X 3502	70	2			1		3
Md. 683 X 3502	77				1		1
3504 X Midland	49	8	1	2			11
3504 X Midland	70					1	1
3504 X 8x A.V.	49	2		3			5
3504-2-2 X Midland	63	11		11	1		23
3266-1-1 X Midland	--	5	6	7			18
3504-2-2 X 3502-1-1	70	3	1		8	8	20
3267-1-1 X 3502-1-1	70	8	2			10	20
3266-1-1 X 3502-1-1	70	1				11	12

The results can be summarized best by examining the crosses according to the parentage groups.

There were only two seedlings obtained from the cross of diploid *F. vesca* x octoploid Midland (Table 2). Both seedlings were pentaploid, weak, and sterile.

Cytological examination disclosed three classes of seedlings from the cross of Midland x tetraploid F.vesca: 18 with 40-42 chromosomes, 8 with 42, and 8 with 56. The first two groups are perhaps all 42-chromosome plants as mentioned previously, but those with 56 chromosomes are apparently from seed that was self pollinated in the flower bud. Since all flowers were emasculated at least a day prior to their opening and were protected from chance pollination after emasculation, the explanation seems to be either precocious pollination through an accidental crushing of an anther or apomixis. To check on this point a cross was made in the spring of 1948 (not shown in Table 3 or 4) using a pistillate octoploid as seed parent and pollen of both the diploid and tetraploid F. vesca. Thirty pollinations yielded only 15 seeds from which were obtained 7 seedlings, none of which were octoploids. Other investigators have reported obtaining maternal type plants in wide species crosses in strawberries (3). Hunter's (10) attempt to induce parthenogenesis in the strawberry resulted in one seedling that he thought may have been a chance pollination.

Most of the hexaploid seedlings were very vigorous and either failed to blossom or were sterile. One seedling out of 26 was slightly fertile (Table 4).

The most interesting crosses were those involving the hybrid hexaploids x cultivated octoploids as it is from these crosses that the decaploids and one eleven-ploid originated (Table 3). Most of the seedlings had the intermediate chromosome number of the parents, but 6 of the 60 hybrid seedlings had 70 chromosomes and 1 had 77. The progeny was too small for the proportion of 6 to 60 to be taken as an exact ratio of what to expect in future work, but it was interesting to have found as many as

6 decaploids in so few plants. These decaploids apparently originated from unreduced gametes of the hexaploids uniting with the regular gametes of the octoploid as follows: 42 from unreduced gamete of hexaploid + 28 from gamete of octoploid = 70. The decaploid seedlings occurred in progenies where the hexaploids were either a seed parent or pollen parent (Table 3) indicating that unreduced gametes were viable both as eggs and as pollen. The meiotic studies give further support by showing that unreduced gametes do occur. The one eleven-ploid plant found must have arisen from an unreduced octoploid gamete uniting with a reduced hexaploid gamete thus: 56 from unreduced gamete + 21 from hexaploid = 77 (Figure 9). The eleven-ploid plant was only partially fertile and was not so vigorous as the decaploids.

Additionally, there were 8 seedlings with 56 chromosomes and, as in the previous cross of Midland x tetraploid F. vesca, it is believed they resulted from precocious self pollination. These were all fully fertile.

In the cross 3504-2-2 x Midland (70 x 56-chromosome parentage) all of the seedlings were apparently enneaploids with 63 chromosomes (Figure 7). They were vigorous and ranged in fertility from plants with no flowers to one with partial fertility, but most of them were only slightly fertile (Table 4). Another similar cross of 3266-1-1 x Midland produced 18 seedlings that showed about the same fertility relationship, but the chromosome numbers were not determined for these as they appeared similar in all respects to the seedlings of 3504-2-2 x Midland. Such a cross probably would be of little value in a breeding program since most of the plants would be too infertile to be of practical use.

Decaploid x decaploid crosses produced seedlings that were apparently all decaploids with 70 chromosomes, considering that those seedlings listed in Table 3 as having 67 to 70 chromosomes probably have 70. The counts of the 70 chromosome plants seemed to be relatively easy to make, perhaps because the large cells made possible fairly flat figures. Although the seedlings were vigorous, their fertility was widely different. Of the 52 decaploid plants, 29 were classified as fully fertile, 8 partially fertile, 3 sterile, and 12 failed to blossom. Fertility in these seedlings obviously was dependent on some factor other than proper chromosome number, but it should be noted that there was a much higher percentage of fertile and partially fertile seedlings in these crosses than in those where the seedlings had 42, 49, and 63 chromosomes.

Meiosis in the polyploid selections

Meiosis was studied in the microsporocytes of the tetraploid F. vesca, the hybrid hexaploid selections 3502 and 4109, the heptaploid selection 3504-2-1, and three decaploid selections 3266-1-2, 3502-1-1, and 3504-2-4. These will be considered separately.

Tetraploid F. vesca. The tetraploid F. vesca usually had some multivalents at metaphase I as would be expected in an autotetraploid. The number and kind of multivalents varied in different cells and no figures were seen that had 14 bivalents. Configurations frequently observed were as follows: 3 quadrivalents and 8 bivalents (Figure 10); 2 quadrivalents, 2 trivalents, and 7 bivalents; 4 quadrivalents and 6 bivalents. Univalents were present in some cases and one cell had 10 univalents plus 3 bivalents and 3 quadrivalents. Regular and irregular metaphase I configurations were

found in microsporocytes from the same anther. Abnormal metaphase behavior in which the chromosomes failed to line up on the plate (Figure 11) was frequently observed. Anaphase I was usually quite regular (Figure 12), but irregular anaphase I configurations were observed. The unequal distribution of chromosomes, as observed in irregular anaphase I, offers an explanation for the occurrence of nuclei at metaphase II having different chromosome numbers and may account for the origin of some of the differences in the size of the pollen. A metaphase II configuration was observed with 16 chromosomes in one group and 12 in the other (Figure 13).

Metaphase II and anaphase II were both regular and irregular in different cells (Figure 14), lagging chromosomes at anaphase II being the most notable feature. An occasional irregular anaphase II was observed where the chromosomes were not grouped at the poles (Figure 15). Although many irregularities were observed, some of the microsporocytes apparently went through meiosis in a regular manner and formed normal-appearing tetrads and pollen.

Hybrid hexaploids. Many meiotic irregularities were observed in the hybrid hexaploids, 3502 and 4109, beginning at diakinesis and continuing through the successive stages to tetrad formation. Univalents, bivalents and quadrivalents occurred at diakinesis. Multivalents and univalents were frequent at metaphase I as indicated by a cell with 4 univalents, 13 bivalents, and 3 quadrivalents (Figure 16). In two other typical PMC the configurations had 3 univalents, 13 bivalents, 3 trivalents, and 1 quadrivalent; and 4 univalents, 9 bivalents, 4 trivalents, and 2 quadrivalents. In numerous cells observed at metaphase I chromosome behavior appeared irregular (Figure 17). Lagging chromosomes frequently occurred at ana-

phase I (Figure 18) accompanied apparently by unequal distribution of chromosomes to the poles. This was reflected in the number of chromosomes found in metaphase II figures as, for example, a cell with 24 chromosomes in one nucleus and 18 in the other (Figure 19). Another cell showed 26 and 16 chromosomes in the nuclei which, as in tetraploid F. vesca, may explain the origin of the differences in size of pollen grains. However, well spread groups of chromosomes at metaphase II were rare and this stage seemed to be characterized by excessive clumping so that accurate counts could not be made in most cells. In one cell 3 groups of chromosomes instead of the customary two were seen at metaphase II. Anaphase II was both irregular (Figure 19) and regular (Figure 20), the latter apparently resulting in normal-appearing tetrads.

Among the tetrads of 3502, there were present cells with only two large nuclei which were apparently dyads (Figure 21). Less frequently monads were seen at this stage (Figure 21). A typical situation is illustrated (Figure 22) in which there was a normal tetrad, a small microspore in the tetrad group, an unreduced cell and a degenerating tetrad. The small microspores occurred rather often at the tetrad stage (Figure 23). Cells were noted in which the nucleoli were spaced as in tetrads, but there had been no cytokinesis (Figure 24). Such cells were rare, but serve to illustrate the irregularities observed in meiosis. Typically normal tetrads (Figure 25) occurred fairly frequently in the hexaploid and these apparently developed into pollen with the regular reduced chromosome number of 21.

Heptaploid selection. The heptaploid selection, 3504-2-1, was very irregular in meiosis and this was reflected in the plant being only partially fertile. Both multivalents and univalents were frequently noted at meta-

phase I. Configurations occurred in which there were 3 trivalents, 12 bivalents and 16 univalents (Figure 26). Another microsporocyte had 2 quadrivalents, 1 trivalent, 17 bivalents, and 4 univalents; a different cell had 6 quadrivalents, 7 bivalents and 11 univalents, and it was evident from examining other metaphase I configurations that the chromosome association was highly variable. This was further reflected in failure of chromosomes to line up at metaphase I and II. Small microspores occurred as in the hybrid hexaploids. Dyads were rare, but tetrads that did occur appeared fairly regular although they were variable in size.

Meiosis in the cultivated strawberry was not studied in the early stages since previous workers had reported it to be very regular. Tetrads were examined of Fairland and they appeared to be regular and uniform.

Decaploid selections. The meiotic behavior of the decaploids varied somewhat depending on the selection involved. Thus 3266-1-2 which had the most aborted pollen (Table 5) showed multivalents at metaphase I and failure of chromosomes to line up properly on the plate (Figure 27). Small microspores were observed, one of which is shown in Figure 33. The other decaploids were quite regular, especially during the early phases of meiosis, with 35 bivalents present at metaphase I (Figure 28), and lined up precisely on the plate (Figure 29). In some of the later stages, however, abnormalities were observed such as an occasional dyad (Figures 30 and 32) and unreduced cells among tetrads (Figures 30 and 31). Some of the unreduced cells had chromosomes at metaphase (Figures 30 and 31) but they were quite different in appearance from typical metaphase I as shown in Figure 29. For the most part tetrad formation was regular, giving a high percent of good pollen in the decaploids.

Pollen studies

Pollen was examined from F. vesca, Blakemore, Fairpeake and the polyploid selections since the kind of pollen produced is a result of and also a reflection of the meiotic behavior of the plants. The percentage of stainable pollen in random samples of the different selections or varieties is given in Table 5 and the variability in size of pollen in Table 6. There was an out-

TABLE 5. Percentage of good pollen in random samples and fertility of plants in strawberry varieties and selections differing in chromosome number

Varieties and Selections	Chromosome Number	Pollen examined No.	Good pollen %	Fertility
Fairpeake	56	702	44.9	Fertile
Blakemore	56	705	61.4	Fertile
F. vesca 2x	14	500	99.0	Fertile
F. vesca 4x	28	974	54.1	Partial
3502	42	436	50.0	Slight
3504-2-1	49	777	36.6	Partial
3502-1-1	70	853	58.7	Fertile
3266-1-1	70	328	71.0	Fertile
3266-1-2	70	466	8.2	Fertile
3267-1-1	70	785	78.1	Fertile
3504-2-4	70	438	82.0	Fertile

standing difference in the appearance of the pollen samples from the various plants (Figures 34 to 39). Aborted pollen was rarely found in diploid F. vesca and its grains were very uniform in size and shape (Figure 34). The tetraploid F. vesca pollen (Figure 35) on the other hand had 54 percent

TABLE 6. Diameters of 100 pollen grains in random samples of varieties and selections differing in chromosome number

Varieties and Selections	Chromosome Number	Diameters in microns and number of pollen grains in each size								
		20	22.5	25	27.5	30	32.5	35	37.5	40
F. vesca 2x	14	91	9							
F. vesca 4x	28	11	9	47	28	5				
3502	42	8	7	27	31	22	3	1	1	
3504-2-1	49	3	27	42	9	11	4	2	2	
Fairpeake	56			28	57	15				
Blakemore	56			45	35	22				
3266-1-1	70				9	47	28	13		3
3266-1-2	70	7	21	57	4	2	1	5	1	2
3267-1-1	70		2	9	18	34	11	23	2	1
3502-1-1	70			1	3	16	30	46	3	1
3504-2-4	70			11	50	39				

that was stainable and it was variable in size (Table 6), indicating differences in chromosome number. The 3502 hexaploid had 50 percent stainable pollen which was extremely variable in size as might be expected with 5 distinct sizes visible in Figure 36. There was an even greater reduction in good pollen (Figure 37) in the 3504-2-1 which is the heptaploid selection that is partially fertile. In the decaploid selections the percent of good pollen was equal to or higher than that of Fairpeake and Blakemore except for 3266-1-2 where the most irregularity in meiosis in the decaploids occurred. Many of the pollen grains of 3266-1-2 were smaller than the other decaploids, but the pollen of all the decaploids was somewhat variable in size with 3504-2-4 being the most uniform (Figure 39).

General characteristics of the polyploid plants

The typical growth habits, size of plants, and fruiting behavior of the different polyploids and parents are illustrated in Figures 41 to 52.

The tetraploid F. vesca plants were usually about as tall as the diploids (see Figure 41) with larger, thicker leaves that were more sharply serrate on the margins (Figure 42) and its flowers were usually larger with broader petals than the diploid (Figure 40). It was much less fertile than the diploid. Seeds of the tetraploid F. vesca were over twice the weight of the diploid -- 66.6 mgms. ± 0.66 per 100 seeds compared to 28.2 mgms. ± 0.44 for the diploid.

The hybrid hexaploids were usually very vigorous, produced abundant runners and were sterile, but a few plants were inclined to be weak. Most of the plants were completely sterile, but a few such as 3502 and 3504 were slightly fertile producing from 1 to 10 or 12 seeds on "nubbin" berries. The foliage of vigorous plants was somewhat like that of the octoploids and was altogether different from F. moschata, the natural hexaploid species by having larger, less rugose leaflets and thicker petioles.

The heptaploid plants had foliage very similar in appearance to that of the cultivated strawberry with much the same growth habit. Most of the plants produced little or no fruit. However, the selection 3504-2-1 was partially fertile and yielded many fruits when cross pollinated, but it was self-unfruitful. Its fruit was somewhat more elongate than that of most cultivated varieties and was aromatic but not so highly aromatic as some of the decaploids.

The appearance of the enneaploid plants in general was similar to that of the heptaploids. Like the heptaploids most of the enneaploids were sterile or only slightly fertile.

The decaploid plants were indistinguishable in foliage and growth habit from cultivated varieties. The seedlings differed in fertility as pointed out previously, but the selections that were made were fertile and prolific. Although the fruit tended to be spongy on most of them, the 3504-2-4 had firm fruit comparable to firm-fruited cultivated kinds and all of the selections had high aroma, since it was one of the characters for which they were selected. Their fruit size was much larger than the tetraploid F. vesca, but usually not so large as the bigger-fruited cultivated varieties.

DISCUSSION

Since previous attempts (5, 7, 8, 11, 12, 15, 18, 19, 21, 26) to incorporate diploid F. vesca with cultivated strawberry failed due to complete sterility of the pentaploid hybrids, it is of interest to point out that use of an autotetraploid F. vesca when crossed with cultivated strawberry has given slightly fertile hexaploids. The tetraploid F. vesca is partially fertile despite extensive multivalency during meiosis, a condition which Fedorova (9) believed caused complete sterility of the tetraploids that she obtained from F. vesca x F. moschata. Even though unequal distribution of chromosomes occurred during meiosis of the tetraploid, the seedlings that were examined of the cross tetraploid x octoploid were all hexaploid. Consequently, it appears that gametes with aneuploid chromosome numbers are non-functional or inviable for the most part or that seedlings fail to grow when there is an unbalanced chromosome condition. Supporting evidence for such an interpretation is found in crosses of the octoploid x hexaploid or reciprocal that produced either heptaploid or decaploid seedlings. Pollen from the hexaploid plants had many different sizes that would indicate

gametes with different chromosome numbers, and it would be expected that seedlings of many different chromosome numbers would occur in the progeny, but such was not the case. This would agree with the results obtained by Fedorova (9) when a partially fertile heptaploid plant when selfed yielded seedlings of a euploid series, but apparently no aneuploids.

The meiotic irregularity of the hexaploid has, no doubt, been an important factor in causing low fertility in these lines. At the same time these irregularities are associated with the production of numerous unreduced gametes which have given rise directly to new fertile types of strawberries. That this non-reduction happens frequently is shown by the occurrence of 6 decaploids in a total progeny of only 60 hybrids from crosses of hexaploid x octoploid or the reciprocal. It seems significant that in the first plants selected for horticultural characters in similar progenies, and without a knowledge of the chromosome constitution, 6 of the 7 plants saved were decaploids originated from unreduced hexaploid gametes uniting with normal gametes of octoploid varieties. When species crosses between different chromosome numbers give hybrids of only slight fertility, it would seem desirable to look for unreduced gametes in the material and plan to utilize them to obtain fertile types. In wide species crosses in strawberry, unreduced gametes occur in material that usually is regular in its meiotic behavior as shown by the eleven-ploid plant found in the cross of octoploid x hexaploid and as noted by other investigators (7, 9, 15, 21). However, these apparently are rare and unpredictable.

The fact that many of the decaploids were relatively fertile suggests that their meiotic behavior was similar to amphidiploids and observations of meiosis seemed to indicate that such was the case. The decaploid plants were composed of 14 chromosomes from F. vesca plus 56 from the cultivated

octoploid, and it seems quite possible that these would behave as amphidiploids, especially since the cultivated octoploids apparently behave as diploids by formation of bivalents at meiosis. However, genetic differences in the chromosome complements of the decaploids plays a part in their fertility as indicated by some decaploid seedlings being sterile or only partially fertile.

Because of the relatively large number of fertile plants in the decaploid class, practical breeding for high aroma probably should be conducted on the decaploid level. In order to have a broad foundation for such work, the tetraploid F. vesca should be crossed with a number of cultivated varieties and a series of hexaploids selected. These in turn should be crossed with a number of cultivated varieties to obtain a series of decaploids with different characters which would then be used as parents in crosses of decaploid x decaploids.

Suggestions and speculations concerning future work along similar lines in strawberries may be of interest to strawberry breeders. Will F. moschata, when crossed with cultivated strawberry, produce unreduced gametes as the hybrid hexaploids do to form decaploids and if so will the plants be fertile? Since it is known that the hybrid hexaploids produce unreduced gametes rather often, a cross of hexaploid x tetraploid F. vesca should give octoploid plants with 28 chromosomes from F. vesca and 28 from the cultivated varieties. These hybrid octoploids perhaps could be used directly in further crosses with cultivated octoploid varieties. Other crosses that would be of interest are hexaploid x decaploid to obtain octoploid seedlings also for crossing with cultivated varieties and hybrid hexaploids x F. moschata (6x) to determine whether the resulting hexaploid seedlings would be more fertile than the hybrid hexaploid parent.

SUMMARY

Cytological studies conducted on strawberry material derived from crosses of a colchicine induced autotetraploid Fragaria vesca and cultivated strawberry gave the following results:

1. The autotetraploid F. vesca had multivalents, bivalents, and univalents present in meiosis, but the plants were partially fertile. When used in crosses with the cultivated strawberry, hexaploid seedlings were obtained some of which were slightly fertile.
2. The hybrid hexaploid plants were very irregular in meiosis and functional unreduced gametes occurred frequently. Such irregularities have been of direct benefit in obtaining new fertile types.
3. The hexaploid plants when crossed with the cultivated octoploids gave seedlings with chromosome numbers of 49, 70, and 77. The 70-chromosome plants originated from unreduced gametes with 42 chromosomes plus the reduced 28 of the cultivated strawberry. The one 77-chromosome plant originated from an unreduced gamete with 56 chromosomes of the cultivated variety and 21 from a reduced gamete of the hexaploid.
4. The 70-chromosome plants (decaploids) are new types which are relatively fertile and some have the high aroma characteristic of F. vesca. These plants have 14 chromosomes from F. vesca and 56 from the cultivated strawberry.
5. Crosses of decaploid plants x octoploids gave seedlings that were enneaploids and the plants were infertile.
6. Crosses of decaploid plants x decaploids yielded decaploid seedlings that were relatively fertile.
7. It is suggested that breeding for improved types with high aroma be done on the decaploid level.

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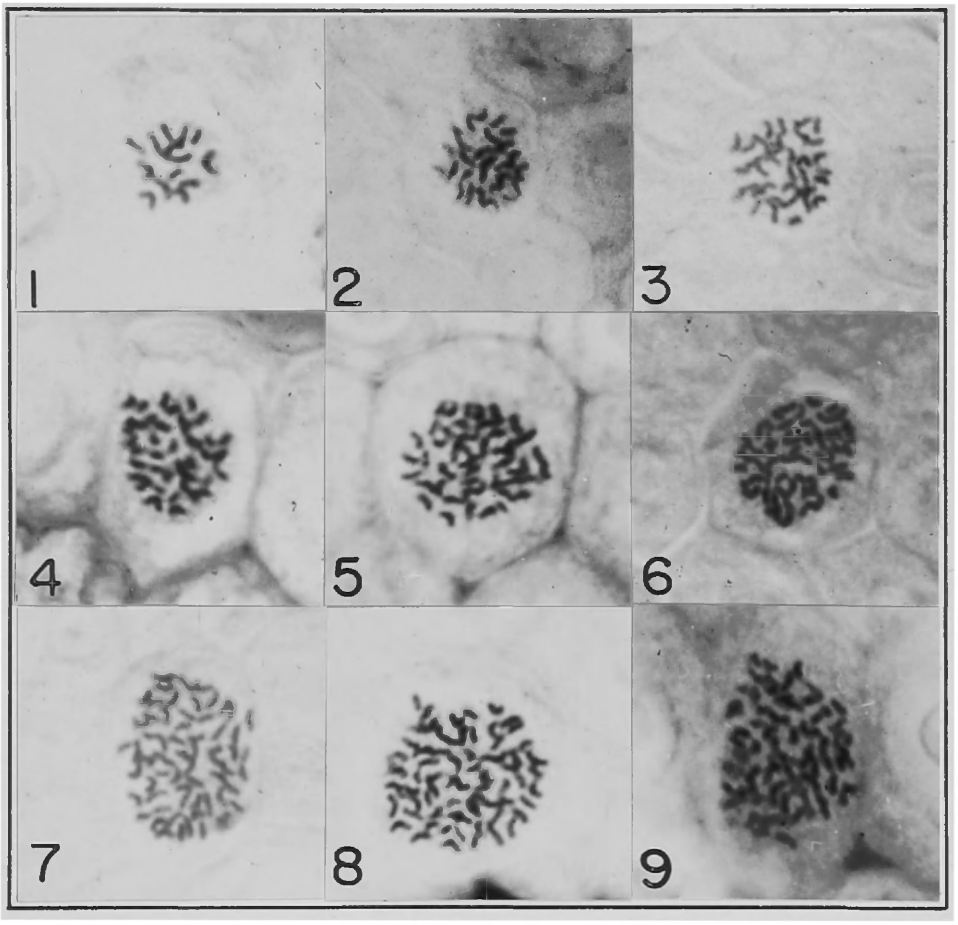
PLATES

Legend for Plate 1

Chromosomes in cells of root tips of *Fragaria* plants

Fig. 1	Diploid *F. vesca*	2n=14
Fig. 2	Tetraploid *F. vesca*	2n=28
Fig. 3	Pentaploid seedling	2n=35
Fig. 4	Hexaploid seedling	2n=42
Fig. 5	Heptaploid seedling	2n=49
Fig. 6	Octoploid seedling	2n=56
Fig. 7	Enneaploid seedling	2n=63
Fig. 8	Decaploid seedling	2n=70
Fig. 9	Eleven-ploid seedling	2n=77

All figures x 2400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Legend for Plate 2

Meiosis in polyploid *Fragaria* plants

Fig. 10 *F. vesca* 4x. Metaphase I with 3 quadrivalents and 8 bivalents

Fig. 11 *F. vesca* 4x. Metaphase I irregular and regular in different cells of same anther

Fig. 12 *F. vesca* 4x. Anaphase I regular

Fig. 13 *F. vesca* 4x. Metaphase II with 16 chromosomes in one group and 12 in the other

Fig. 14 *F. vesca* 4x. Metaphase II regular and anaphase II with 1 chromosome lagging

Fig. 15 *F. vesca* 4x. Late anaphase II irregular with failure of chromosomes to group at the poles

Fig. 16 Hybrid hexaploid. Metaphase I with 3 quadrivalents, 13 bivalents and 4 univalents

Fig. 17 Hybrid hexaploid. Metaphase I with lagging chromosomes

Fig. 18 Hybrid hexaploid. Anaphase I with lagging chromosomes in 2 microsporocytes

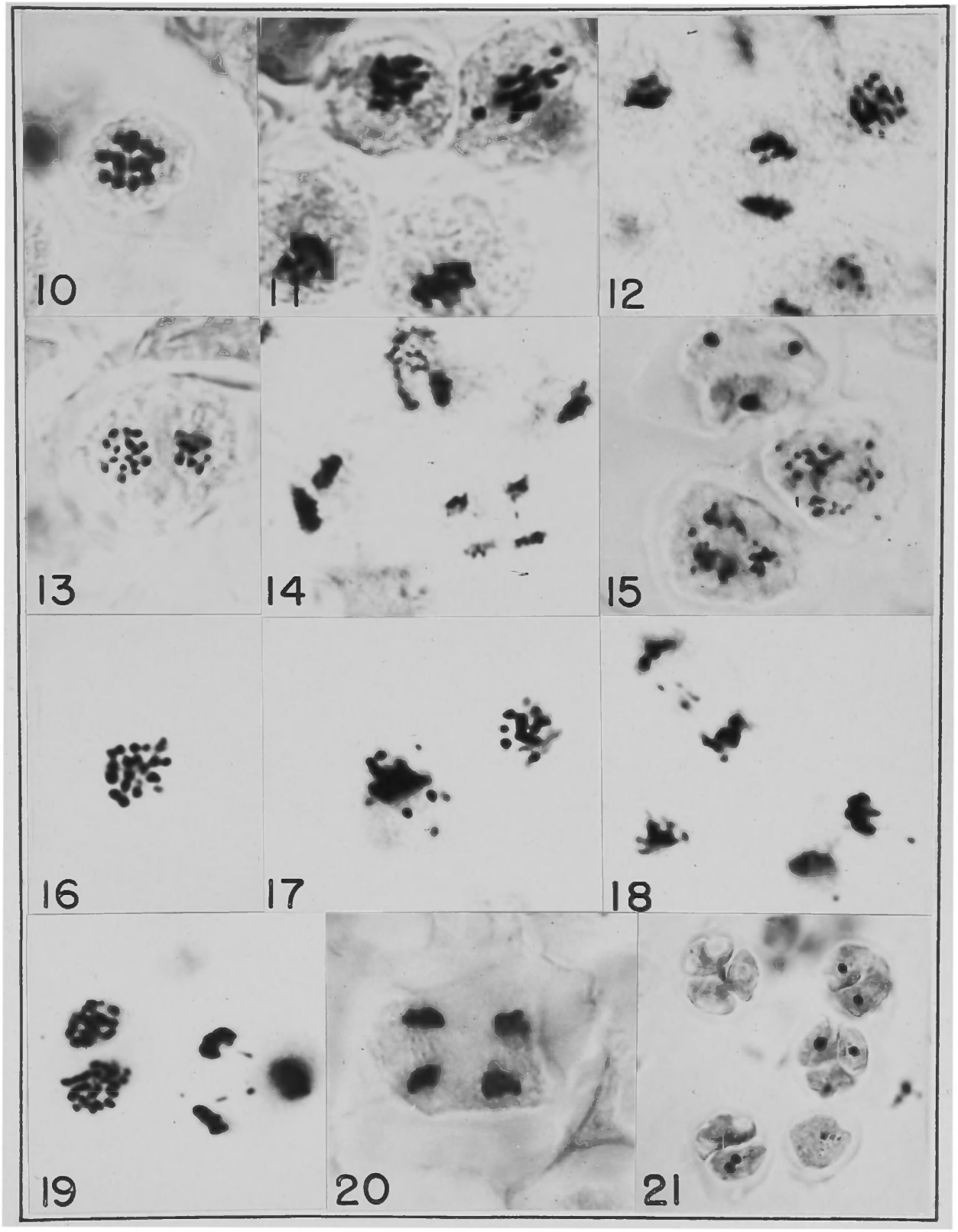
Fig. 19 Hybrid hexaploid. Metaphase II with 18 chromosomes in one group and 24 in the other; anaphase II with lagging chromosomes

Fig. 20 Hybrid hexaploid. Regular anaphase II

Fig. 21 Hybrid hexaploid. Monad, dyad, and regular tetrads

Fig. 10 to 20 x 2400

Fig. 21 x 1350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Legend for Plate 2 - Continued

Fig. 22 Hybrid hexaploid. Small microspore at tetrad stage, a monad, a degenerating tetrad and a regular tetrad

Fig. 23 Hybrid hexaploid. Smear preparation of tetrad stage with 3 large microspores and 2 small microspores

Fig. 24 Hybrid hexaploid. Cell with nucleoli spaced as in tetrads but no cytokinesis

Fig. 25 Hybrid hexaploid. Regular tetrad formation

Fig. 26 Heptaploid selection 3504-2-1. Metaphase I with 3 trivalents, 12 bivalents and 16 univalents

Fig. 27 Decaploid selection 3266-1-2. Metaphase I irregular

Fig. 28 Decaploid selection 3504-2-4. Metaphase I regular with 35 bivalents

Fig. 29 Decaploid selection 3502-1-1. Regular orientation of chromosomes at metaphase I

Fig. 30 Decaploid selection 3504-2-4. Dyad, tetrad, and two monads--one with chromosomes at metaphase

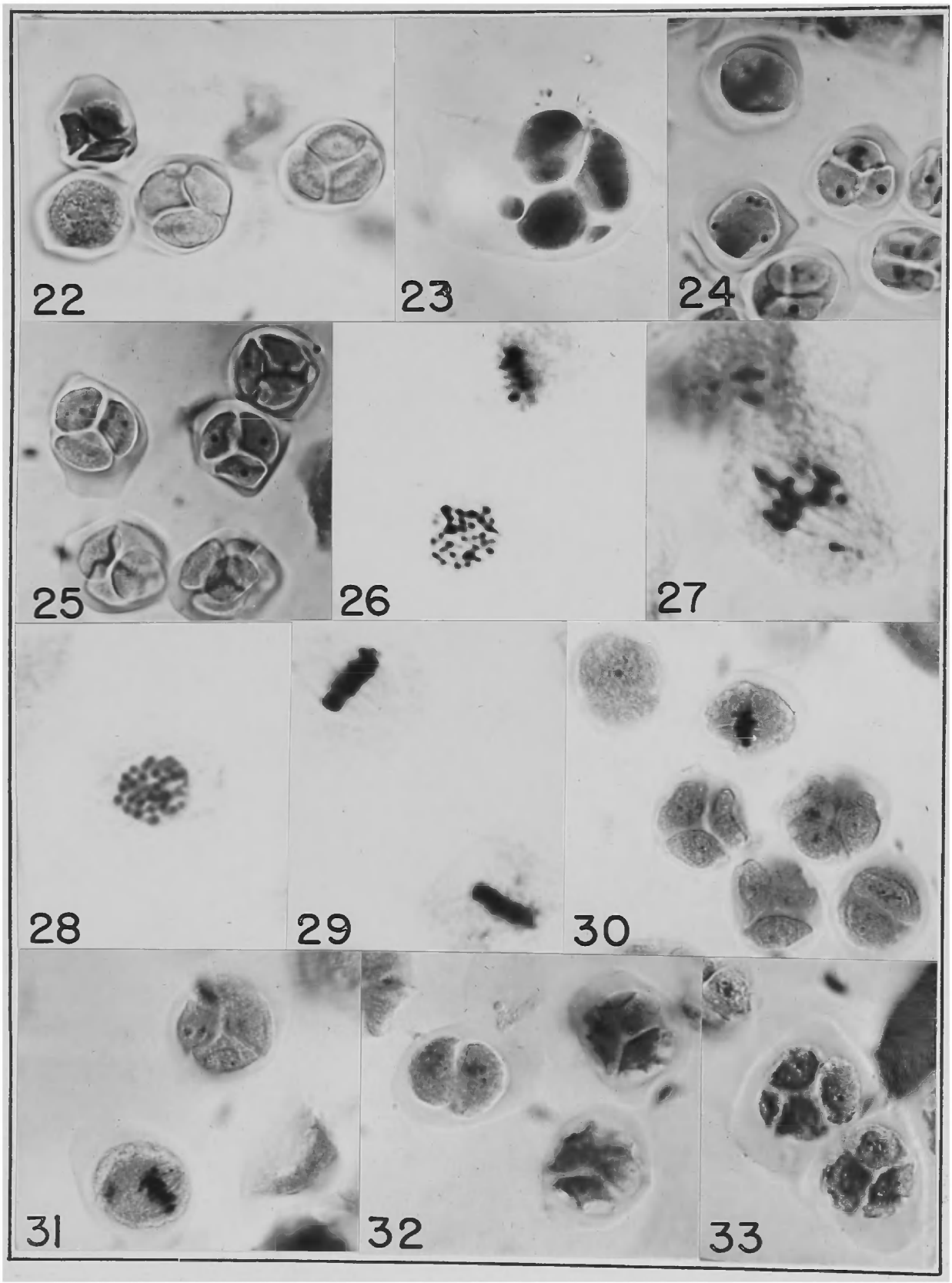
Fig. 31 Decaploid selection 3504-2-4. Monad with most of chromosomes at metaphase, but a small clump of chromosomes on left side of cell

Fig. 32 Decaploid selection 3504-2-4. Dyad and regular tetrads

Fig. 33 Decaploid selection 3266-1-2. Small microspore at tetrad stage

Fig. 22 to 25 and 30 to 33 x 2400

Fig. 26 to 29 x 1350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Legend for Plate 3

Pollen grains and flowers of *Fragaria* plants

Fig. 34 Diploid *F. vesca*. Pollen with uniform size and shape and no aborted pollen

Fig. 35 Tetraploid *F. vesca*. Pollen with different sizes and many aborted grains (unstained)

Fig. 36 Hybrid hexaploid 3502. Pollen with 5 different sizes and many aborted grains

Fig. 37 Heptaploid 3504-2-1. Pollen scarce, variable in size with some very large grains, some aborted grains.

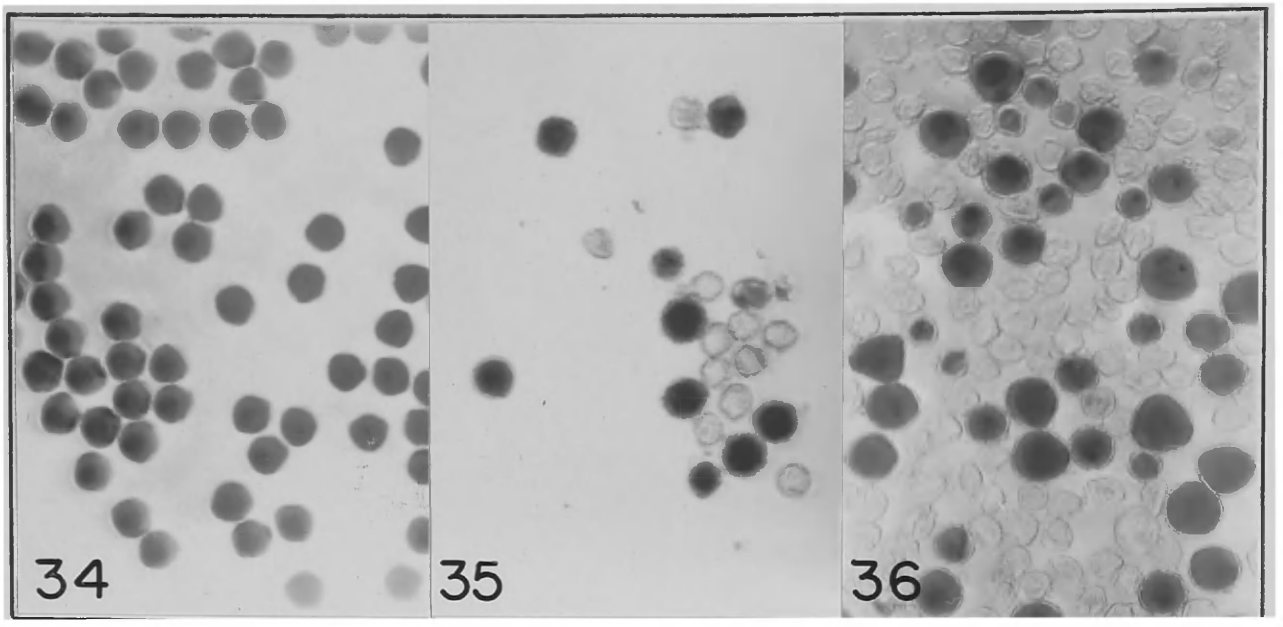
Fig. 38 Octoploid Blakemore. Some aborted pollen

Fig. 39 Decaploid 3504-2-4. Pollen somewhat variable in size, but relatively few aborted grains

Fig. 40 Diploid *F. vesca* flowers on left, tetraploid *F. vesca* on right.

Fig. 34 to 39 x 300

Fig. 40 Natural size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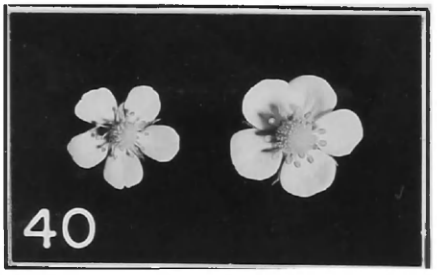
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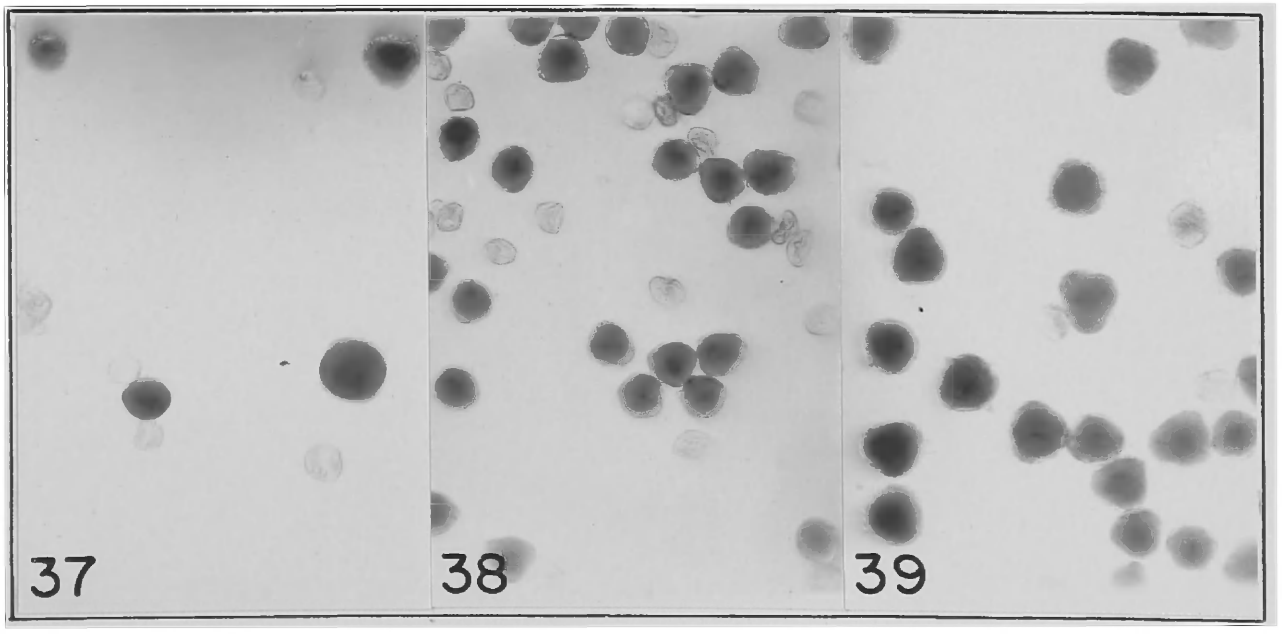
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Legend for Plate 4

Plants of *Fragaria* in a polyploid series

Fig. 41 Diploid *F. vesca*

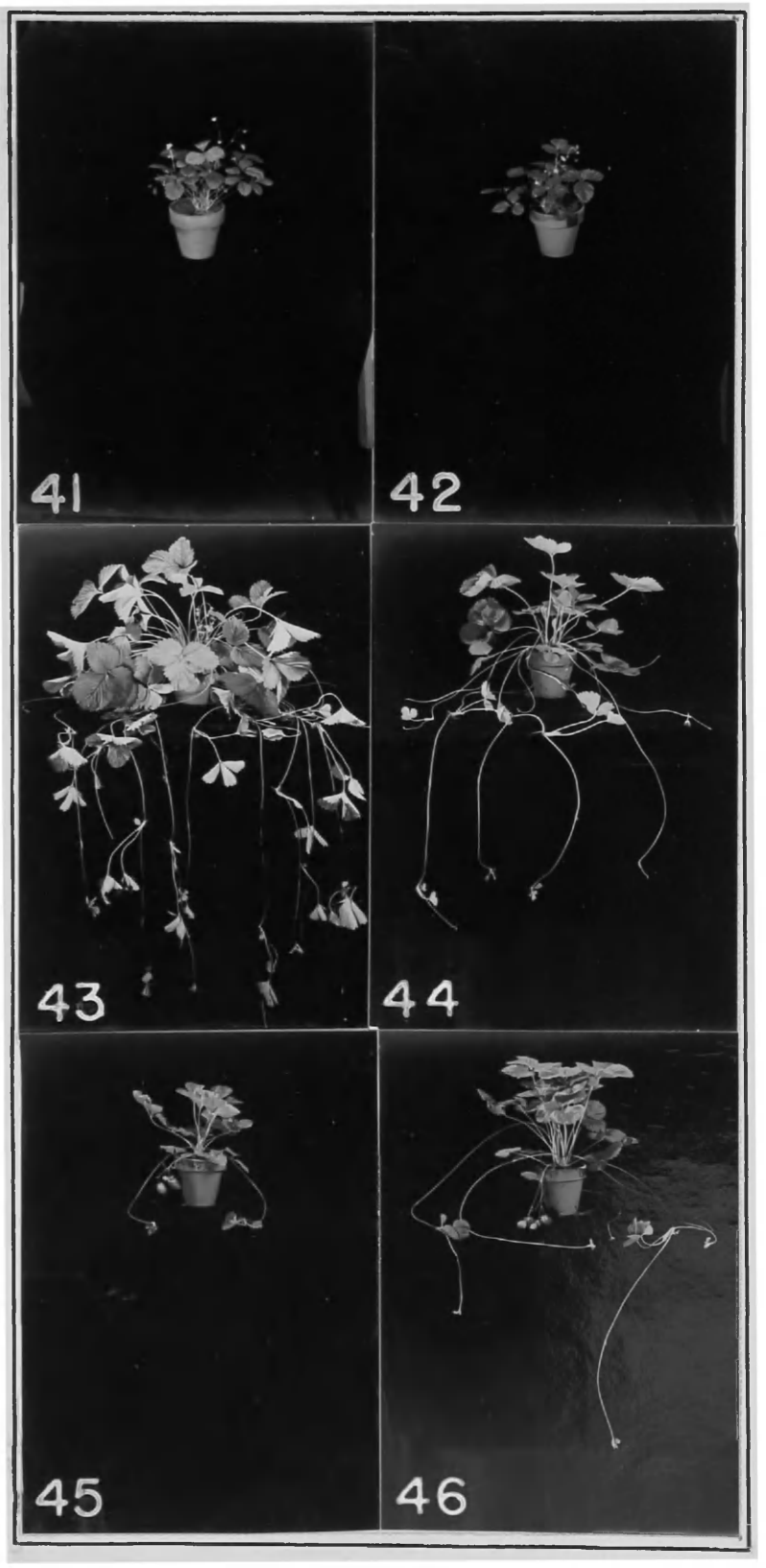
Fig. 42 Tetraploid *F. vesca*

Fig. 43 Hexaploid hybrid

Fig. 44 Heptaploid hybrid

Fig. 45 Octoploid seedling

Fig. 46 Decaploid seedling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Legend for Plate 5

Fruit and leaves of *Fragaria* in a polyploid series

Fig. 47 Diploid *F. vesca*

Fig. 48 Tetraploid *F. vesca*

Fig. 49 Hexaploid selection 3502


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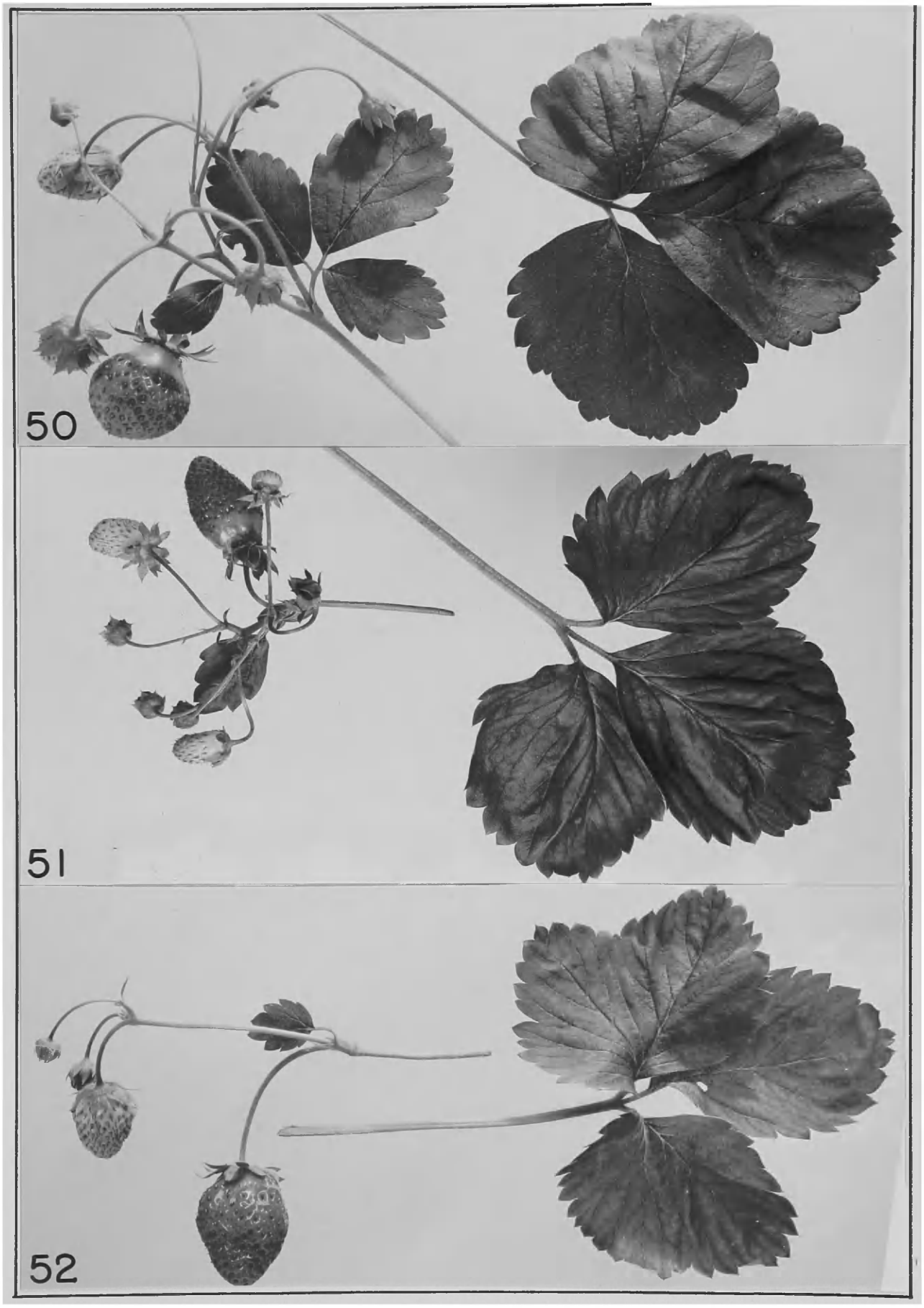
Legend for Plate 5 - Continued

Fig. 50 Octoploid seedling of Midland

Fig. 51 Decaploid selection 3266-1-2

Fig. 52 Decaploid selection 3504-2-4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